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User's Manual

BSA ELISA Kit 2 (Quantitative)

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The BSA quantitative detection enzyme-linked immunoassay kit is designed for the accurate measurement of residual Bovine Serum Albumin (BSA) in various biological products, including intermediates, semi-finished, and final products.

General Description

This specialized ELISA kit features a high-concentration standard solution, catering to the personalized needs of users. Moreover, A 20× Buffer is included which can be used as sample diluent, detection antibody diluent, and wash solution, streamlining the process and ensuring ease of use. The ELISA kit is suitable for direct sample analysis, demonstrating excellent reproducibility and high stability.

Principles of Testing

The ELISA kit utilizes a double-antibody sandwich enzyme-linked immunosorbent assay technique to detect minuscule traces of BSA residue in the sample. The capture antibody is immobilized onto a 96-well plate, creating a solid-phase antibody. Then standard samples and test samples are added, and this is followed by the addition of the detection antibody labeled with horseradish peroxidase (HRP), resulting in the formation of a solid-phase antibody-BSA-detection antibody sandwich complex. After incubation the wells are washed, and a substrate is then added to initiate a color reaction. Under the influence of HRP, the substrate transitions from colorless to blue, and finally to yellow upon the addition of the stop solution. The absorbance values (OD values) are measured at 450 nm and 630 nm wavelengths, with 630 nm serving as the reference wavelength.

Reagents And Materials Provided

1. Microplate: 96 well polystyrene microplate (12 strips of 8 wells).
2. Standard: 1×300 µL, 5 µg/mL.
3. Detection Antibody: 1×40 µL, antibody conjugated to horseradish peroxidase (HRP) with preservatives.
4. Buffer Concentrate 20×: 30 mL of a 20-fold concentrated solution.
5. Chromogen Solution A: 8 mL, avoid direct light.
6. Chromogen Solution B: 8 mL, avoid direct light.
7. Stop Solution: 15 mL.
8. 3 Cover foil.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm
2. Incubator 37°C

3. Shaker
4. Precision pipettes to deliver 0.5 µl to 1 mL volumes
5. Absorbent paper
6. Distilled or deionized water
7. Log-log graph paper or computer and software for ELISA data analysis
8. Tubes to prepare the positive control or sample dilutions

Storage

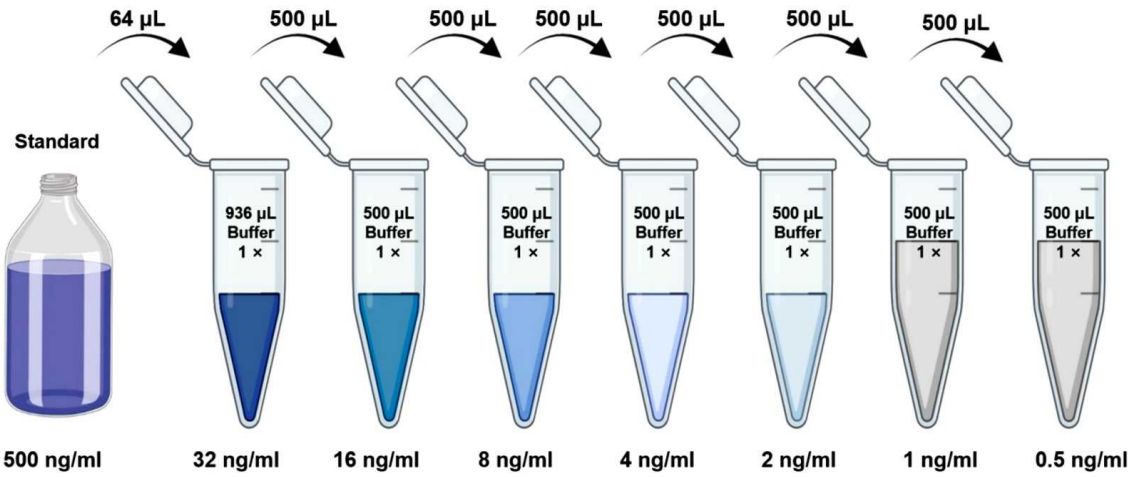
1. Store all reagents at 2-8°C, and not be frozen or thawed. The product is valid for 12 months.
2. All reagents must be brought to room temperature (20-25°C) prior to use.

Specimen Collection And Preparation

Equilibrate the samples to room temperature and mix before loading. If you need to dilute samples or the provided high-concentration standard solution, Buffer (1×) can be used for dilution. For cell samples, it is recommended to centrifuge at 3000 rpm for 5 minutes at room temperature, and then use the supernatant for testing before detection.

Reagent Preparation

1. Buffer (1×): Prepare by diluting Buffer (20×) with deionized water at a 20-fold ratio, for example: mix 10 mL of Buffer (20×) with 190 mL of deionized water. Note: If crystals form in Buffer (20×), gently shake at room temperature or in a 37°C water bath until completely dissolved before dilution.
2. Chromogenic Solution: Mix equal volumes of Chromogenic Solution A and Chromogenic Solution B, then store away from light. Note: Do not store for too long; typically, prepare within 10 minutes before use. Do not use if the chromogenic solution turns blue after mixing.
3. Detection Antibody (1×): Remove the Detection Antibody from the refrigerator, and dilute it 500-fold with Buffer (1×) according to the experimental amount (100 µL/well) to obtain the Detection Antibody (1×). Note: Do not leave the enzyme-labeled antibody at room temperature for an extended period; it's best to use it immediately after removal from the refrigerator.
4. Standard Solution: Prepare a new standard solution for each experiment. Add 7 µL of 5 µg/mL standard solution to 63 µL of Buffer (1×) to obtain a 500 ng/mL standard solution. Then, add 64 µL of 500 ng/mL standard solution to 936 µL of Buffer (1×) to dilute it to 32 ng/mL. Subsequently, dilute it according to the 2-fold ratio as shown in the figure below:



Assay Procedure

1. Allow all components of the ELISA kit to equilibrate at room temperature for 30 minutes. Remove the required strips from the aluminum foil bag, and mark the strip sequence with a marker. Note: The strips are prone to detachment during the washing process, so Careful marking is important.
2. Sample Incubation: Add diluted standard solutions and test samples to the microplate (recommended: standard wells, blank wells and sample wells), 100 µL per well. Subsequently, add Detection Antibody (1×) to each well, 100µL per well, seal the plate with plate sealer film, and then incubate at 37°C with shaking at 400-600 rpm for 1 hour. Note: Control the sample should be added within 10 minutes to avoid time-dependent drift. Incomplete sealing or lack of sealing during incubation can lead to evaporation of the reaction solution, causing experimental errors.
3. Plate Washing: After incubation, carefully remove the plate sealer film, discard the liquid from the wells, wash the plate three times with Buffer (1×) (250 µL per well), and then pat dry to remove residual liquid from the sample wells. Note: If washing manually, allow the plate to rest for 1 minute after adding Buffer (1×); if using a plate washer, gently shake for 5 seconds after adding Buffer (1×).
4. Chromogenic Reaction: Add the pre-prepared chromogenic solution to the microplate, 100 µL per well, seal the plate with plate sealer film, and incubate at 37°C in the dark for 20 minutes.
5. Termination: Add the stop solution, 100 µL per well, and read the results after even color development (Note: Reading should be completed within 20 minutes of adding the stop solution).
6. Reading: Place the microplate in the microplate reader, set the wavelength to dual wavelength (450/630 nm), and read the absorbance values (Note: It is recommended to set 5-10 seconds of shaking in the microplate reader program).

Calculation

1. The calculation of the absorbance correction value for each standard or sample is:

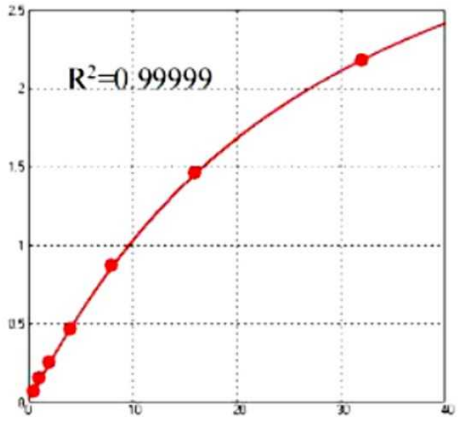
   OD_{450nm} - OD_{630nm} - $OD_{Blank\ Control}$
2. Plot a standard curve with the concentration of the standard as the X-axis and the absorbance correction value of the standard as the Y-axis. It is recommended to use the four-parameter logistic mathematical model fitting equation:

Y = ((A-D)/(1+(X/C)^B)) + D

Substitute the absorbance correction value of the sample into the formula to calculate the content of BSA in the sample. (Pay attention to the dilution factor).

3. If the OD value of the sample exceeds the highest OD value on the standard curve, the sample needs to be diluted and retested.
4. Recommended dual-wavelength correction method. Use a wavelength of 630 nm for correction. The corrected OD value is OD_{450} - OD_{630}. This corrected value can be used directly for calculation or, based on data quality, further blank correction can be performed. If a dual-wavelength microplate reader is not available, after reading the OD_{450} data, the data quality should be evaluated before performing blank correction.

Typical Standard Curve



STD (ng/mL)	OD
32	2.1802
16	1.4573
8	0.8665
4	0.4642
2	0.2535
1	0.1505
0.5	0.0684

Performance Characteristics

Intra-assay Precision (Precision within an assay): CV% < 10%

Inter-assay Precision (Precision between assays): CV% < 15%

Spike Recovery: 70% - 130%

Detection Range

1.56 - 50 ng/mL

Detection Limit

< 1 ng/mL

Sensitivity

1.56 ng/mL

Specificity

Horse Serum < 1%

Rabbit Serum < 1%

Collagenase Type I < 1%

Cysteine < 1%

Benzonase® Nuclease < 1%

Trypsin analog TrypLE™ < 1%

Precautions

1. The container used for determining the BSA content should be dedicated to prevent BSA contamination in the laboratory.
2. The background value of this kit (i.e., OD 0 ng/mL before correction) is generally not greater than 0.2. If the background value exceeds 0.2, an investigation into the environment, containers, and consumables is necessary.
3. All components of the reagent kit must be equilibrated to room temperature (20-25°C) before use.
4. Before using each component, thorough mixing is required to ensure the uniformity of the reagent. Standard solutions also need to be briefly centrifuged for 5 seconds to concentrate any liquid from the walls and lid into the bottom of the tube. All reagents should be returned to 2-8°C immediately after use.
5. The reagent kit must be used within its shelf life. A corresponding standard curve needs to be prepared for each experiment, and it is not recommended to mix and use related reagents from different batches.
6. When adding liquid to the microplate, care should be taken to avoid touching the bottom of the microwell plate to prevent damage to the coating layer. Different samples and steps require timely replacement of the sample loading slots and tips to avoid cross-contamination.
7. When patting dry after washing the strips, attention should be paid to preventing the strips from falling off, and the plate sealing film should not be reused.
8. When the residual concentration of the target to be tested is too high, the coloration process may produce black flocculent. In such cases, the sample needs to be diluted to a certain extent before testing.
9. During reading, ensure that the detection wavelength and fitting equation selection are correct.
10. Strict adherence to the operational methods specified in the instructions, and the exclusive use of reagents provided with this kit, are essential to ensure optimal detection results.
11. Different test results can be caused by various factors, including the operator's technique, pipetting, plate washing technique, reaction time or temperature, and the storage of the reagent kit.
12. Our company is only responsible for the reagent kit itself and is not responsible for sample consumption resulting from the use of this kit. Users should consider the likely amount of sample to be used and reserve an adequate amount of sample before use.
13. This ELISA kit is for in vitro research use only and is not intended for clinical diagnosis.